CRP: Livestock

Flagship: FP3 – Feeds and Forages

Cluster: 2 - Development of new feed and forage options

Activity: P1685 – Activity/Product Line 3.2.1: Improved feed & forage germplasm and new tools and technologies for breeding

Deliverable: D17251 - Report on phenotyping of the hybrid progenies and identification of the best male tester as well as magnitude of heterosis and hybrid performance for population Pm20

Report of Seed quality in two *Urochloa* collections

David Alberto Florián Vargas, Agronomic Engineer.

ABSTRACT

In order to deliver to donors the hybrid materials that have already been preselected and that are about to be evaluated for commercial release, a count was made of the botanical seed that was available for said delivery. Once the quantities of seed were available, an evaluation process of its quality characteristics was continued, taking into account parameters such as the age of the seed (from the time of harvest to the present), percentage of germination and percentage of viability. Carrying out scarification procedures and the respective necessary germination (Germination in substrate) and viability (Tetrazolium method) tests. Finally, the information obtained was analyzed using descriptive statistics and presented in frequency bar diagrams, in which classifications were made by class, placing the hybrids to be delivered to the donor in each of these classes.

INTRODUCTION

Botanical seed quality, in the case of tropical forages, is not an attribute that should be considered in isolation from production (López, NA, González, AO, Ruz, MR, & López, LL, 1975), since to ensure the Establishment of future pastures, must be closely related to variables such as the percentage of germination, viability and storage time, which together provide a global idea of the quality of a group of seeds. Of these variables we can give definitions to be able to understand seed quality in a more specific way, starting with germination, which is defined as the adequate development and emergence of the structures that make up the embryo, to give rise to a new plant (López, NA, González, AO, Ruz, MR, & López, LL, 1975), a concept that is widely related to viability, which speaks of the ability of a batch of seeds to germinate under favorable environmental conditions (García, FP, & Villamil, JMP, 2001).

For its part, storage time is related to the longevity of the seeds, which is defined as the time that a seed can remain viable, under ideal conditions of temperature and humidity (García, FP, & Villamil, JMP, 2001). Analyzing these variables is important, since the seed that is being used for this study corresponds to

hybrids obtained in CIAT's tropical forage improvement program, said improved materials are destined to be commercialized by private sector companies, which are responsible for the production of seed and its subsequent distribution, as is the Papalotla Group, a Mexican company dedicated to this work (Hare et al., 2007), which, through alliances with the International Center for Tropical Agriculture - CIAT-, has made effective the release of different Urochloa Hybrids, such as Mulato, Mulato II, Cobra and Cayman, which have outstanding characteristics in terms of dry matter yield, adaptability, resistance to pests and nutritional value (Martinez, J. RG. Et al., 2020); which is desirable for the production of forages in tropical areas with diverse edaphoclimatic characteristics (González B., 2013).

The analyzes were made on the conditions that keep the seeds in two collections of hybrids, one of them is the Br12 group (*Urochloa interspecific*, generation of 2012), which would be the next in the line of hybrids; and the Bh16 group, a group improved *Urochloa humidicola* (generation of 2016), to make these shipments first the availability and quality of the botanical seed is reviewed, addressing for this last parameter, the germination percentage, harvest time and viability that said seeds present (Pérez, A ., & Febles, G., 1988), understanding that this set of variables will allow us to understand in a global way the seed quality of the hybrid groups, this being the objective of this research.

MATERIALS AND METHODS

The study was carried out at CIAT headquarters, located at Km 17, of the recta Cali - Palmira, in Colombia, using the facilities of the tropical forage genetics laboratory and the tropical forage genetics greenhouse. The seed was stored in the Laboratory in a cold room, which maintains a temperature of 8 °c and a RH of 50%, said seed is stored in plastic and / or cardboard envelopes that have the identification of the corresponding genotype, in this case the seed corresponds to two collections of hybrid *Uroclhoa*, the Br12 collection harvested in 2013 and the Bh16 collection harvested in 2017.

To capture the seed availability of the two hybrid groups, the envelopes were taken one by one and the seed content was weighed in a precision scale sartorus brand, these data were recorded together with the other variables evaluated in tables 1 and 2, in many of the cases there were several envelopes belonging to the same hybrid, for which the total available weight was reported in the table; after this the seed was returned to the envelope and stored again in the cold room (See Figure 1).

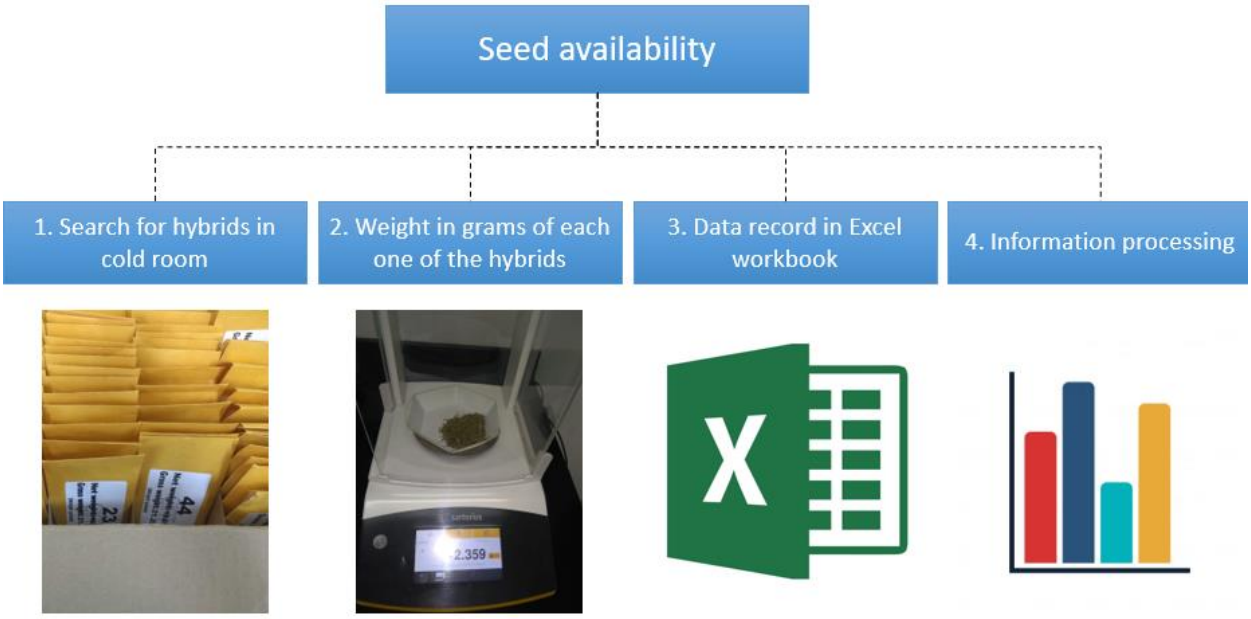


Figure 1. Urochloa seed availability determination

The germination percentage was determined by performing a germination test in silica sand, which is a variant of the standard germination test, including a substrate (ISTA, 1999), as shown in figure 2. This has been tested in CIAT's tropical forages program, as it is known that this germinative substrate facilitates the speed of seedling development (Natera, JRM, Moreno, MJ, & amp; Moya, JF, 2009). The test was carried out with seed scarified with sulfuric acid at 96% H2SO4 (García, J. and Cícero, SM 1992), placing the seeds in a beaker for a time of 15 minutes, the seeds were constantly shaken, then neutralized the acid by washing with abundant water and finally they were dried on a tray with paper at room temperature, as shown in figure 3; These scarified seeds that were subjected to a germination test, began to be evaluated 8 days after sowing, with the data obtained, the percentage of germination of the hybrids is calculated applying the following equation:

Equation 1. Calculation of the percentage of seed germination.

$$\%\ Germination\ = \frac{number\ of\ germinated\ seeds\ x\ 100}{Total\ number\ of\ seeds}$$

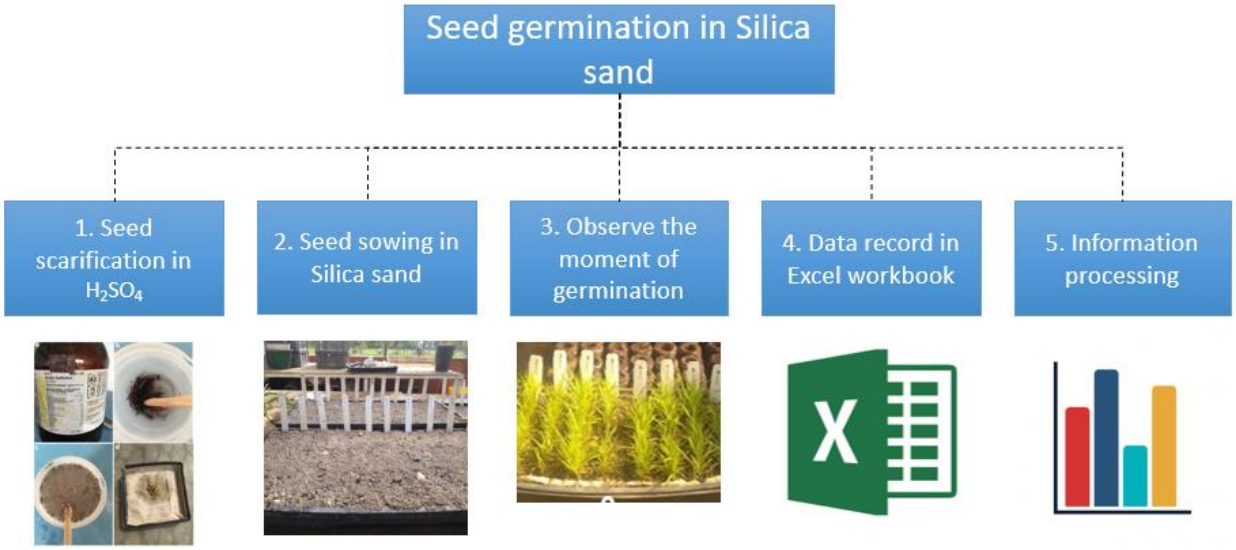


Figure 2. *Urochloa* seed germination test in Silica sand.

Figure 3. Scarification of *Urochloa* seed with 96% H_2SO_4, a) Sulfuric Acid concentration of 96%, b) Agitation of seeds for 15 min, c) Neutralization of the acid with water, d) Seeds dried on paper at room temperature.

Viability was determined by the tetrazolium test, the methodology used in this determination consisted of leaving the seeds (scarified with H2SO4 in the case of Br12 and manually in the case of Bh16) in imbibition at room temperature for a period of 24 h, then they were longitudinally bisected through the embryonic axis; One half was placed in the 1% Tetrazolium solution for 3 hours at 40 ° C and the other half was discarded (see figure 4). The bisected caryopses were washed with water and evaluated for viability using the guide described by Ruiz, MA (2009), in Figure 5, seeds that were subjected to tetrazolium staining

are visualized, with the data obtained the percentage of the hybrids (as indicated in equation 2) and these data are reported in Table 1.

Equation 2. Calculation of the percentage of viability of seeds

$$\%\, Viability\ = \frac{Number\ of\ seeds\ dyed\ x\ 100}{Total\ number\ of\ seeds}$$

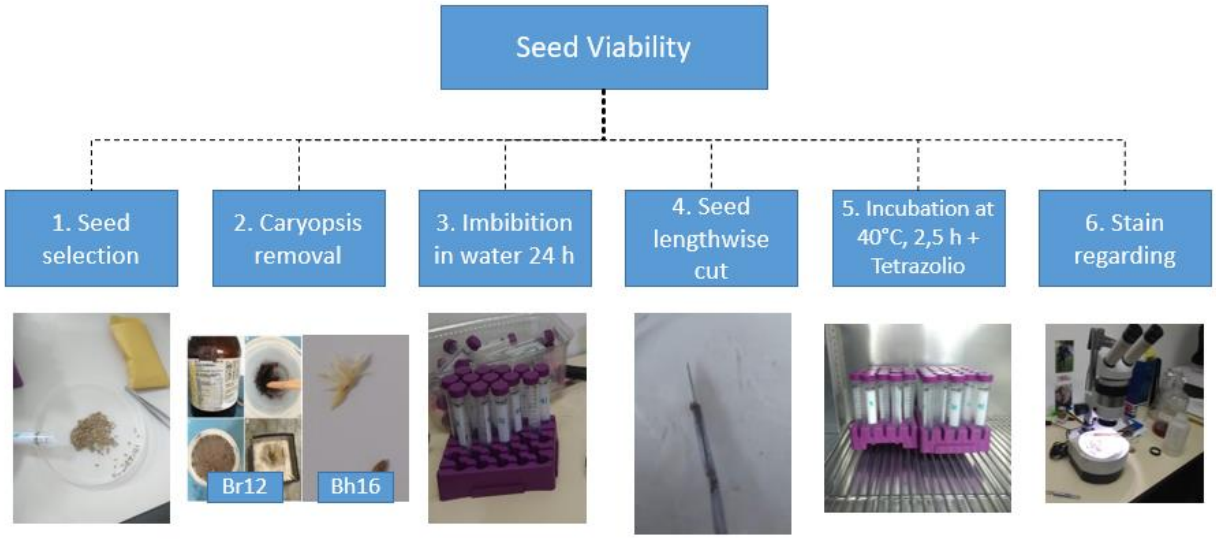


Figure 4. Urochloa Seed Viability Test in Silica sand.

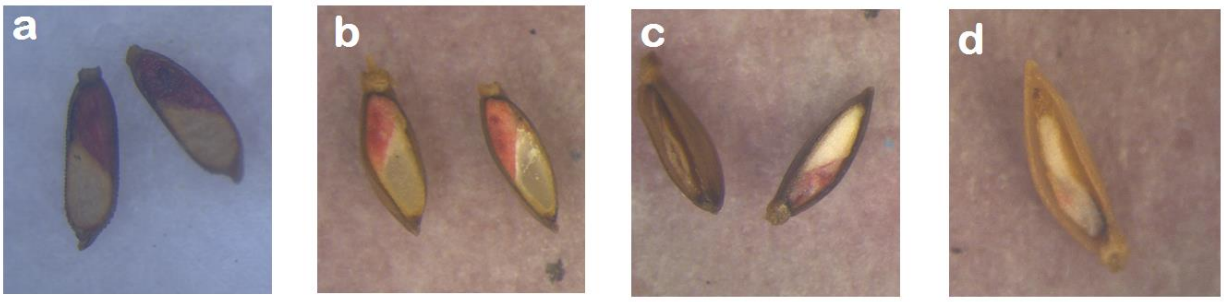


Figure 5. *Urochloa* Seed Viability Reading a) Completely Viable Seed, b) Viable Seed that will probably germinate with little Vigor, c) Seed considered unviable, d) Inviable Seed - dead.

Finally, the data obtained were processed in Microsoft Excel, elaborating histograms of classes in which the levels of germination, viability, and seeds available for shipment are reported; additionally, in table 1, the dates on which the hybrids were harvested are reported to complete the pertinent information to the seed group.

RESULTS Y DISCUSSION

Based on the variables evaluated, the following table was constructed, in which the status of the hybrid groups Br12 and Bh16 is summarized in a class system, including the variables Seed availability, Germination percentage, Viability percentage and Date harvest, including the mean and standard deviation for the variables of the two hybrid groups.

Table 1. Variables evaluated to determine the seed quality of the hybrid groups Br12 and Bh16.

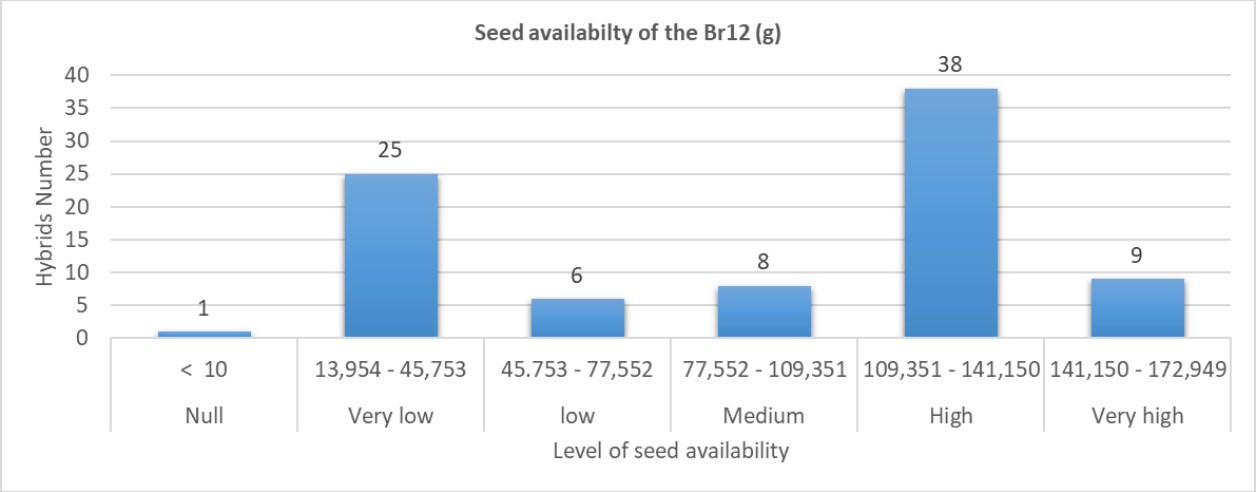
Hybrid group	Class	Evaluated Variables			
		Seed availability	% Germination	% Viability	Harvest date
		Number of hybrids per class			
Br12 (87 hybrids)	Null	1	1	1	August - October 2013
	Very low	25	42	23	
	low	6	32	46	
	Medium	8	12	17	
	High	38	0	0	
	Very high	9	0	0	
Average		*90,6*	*23,5*	*31,7*	
Estandard dev.		*49,4*	*14,7*	*14,3*	
Bh16 (27 hybrids)	Null	9	3	5	August - September 2017
	Very low	12	3	0	
	low	2	7	3	
	Medium	3	9	5	
	High	0	3	9	
	Very high	1	2	5	
Average		*10,9*	*41,5*	*55,2*	
Estandard dev.		*14,1*	*25,2*	*31,6*	

** The intervals of the classes can be viewed in graphs 1,2,3,4,5 and 6.

Next, the variables analyzed with the histograms elaborated from the collected data are reported, clarifying that the frequencies of the classes were established in the following way: For the available seed, the width of the interval was calculated subtracting the maximum value reported from the value smaller, then the difference was divided by 5, this value being the width of the interval; 0 was assigned as a class the name "Null", for the variables Germination and Viability, being percentages the class "Null" was established for 0% and the remaining 5 classes present amplitudes of 0.2 or 20% until completing 1 or the 100%.

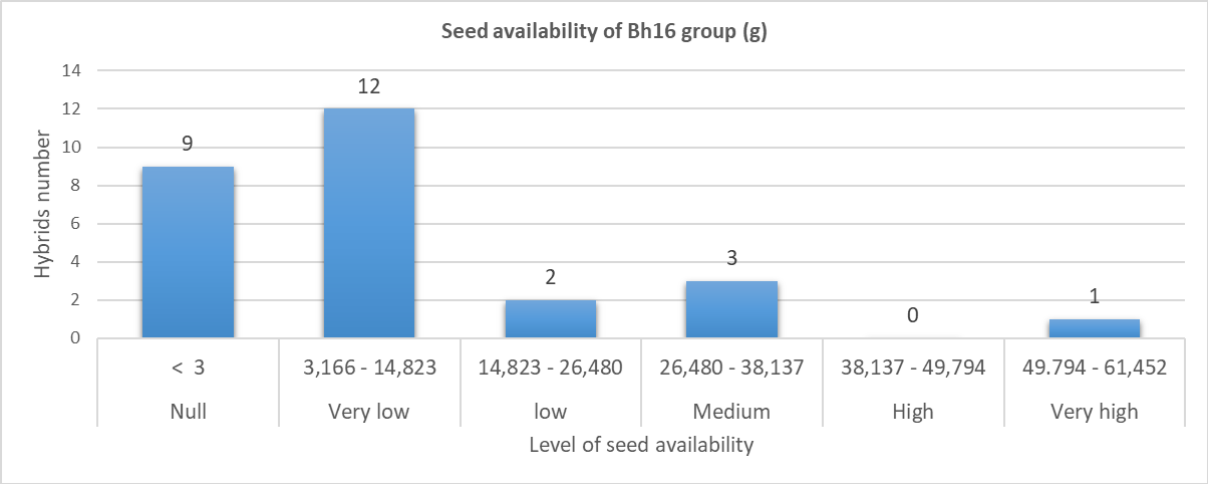
Seed disponibility

For the Br12 group, all the seed are duly identified and scarified with Sulfuric Acid, there is an acceptable number of hybrids with seed available (55 out of 87), taking into account that it is planned to send all the available seed being the hybrid with the least amount of seed is Br12 / 3136 with 4.3 g. of seeds, although the technician's recommendation is to send 20 g / envelope, each gram has an average of between 150 and 180 seeds and an average value of 90.6 g per hybrid. In the case of group Bh16, no scarification procedure has been carried out, there are few hybrids with medium or high levels of seed available (4 of 27), taking into account that it is planned to send sachets with at least 6 g. of seeds (due to its low availability), although the technician's recommendation is 20 g / envelope, each gram of seed has an average of between 220 and 250 seeds and an average value of 10.9 g.



Graph 1. Available seed of the Br12 group hybrids.

Observing Graph 1, it is evident that there is enough seed to send the 87 that make up the group, from this it is presumed that for 25 hybrids between 10 and 40 grams can be sent with botanical seed, for 6 hybrids it can be sent between 40 and 70 grams with botanical seed, for 8 hybrids between 70 and 100 grams can be sent with botanical seed, for 38 hybrids between 100 and 140 grams with botanical seed can be sent and for 9 hybrids between 140 and 170 grams can be sent with botanical seed.

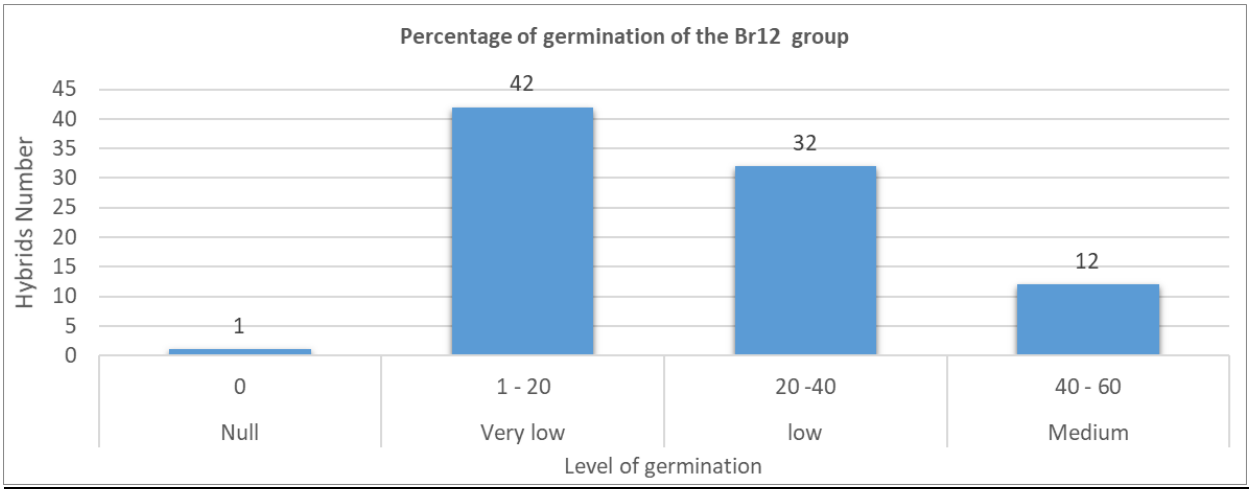


Graph 2. Available seed of the hybrids of the Bh16 group

Observing Graph 2, it is evident that there is little seed available, since only approximately 15 hybrids of the 27 that make up the group can be sent, adding that for most hybrids (12) only between 3 and 14 g of seed could be sent.

Germination

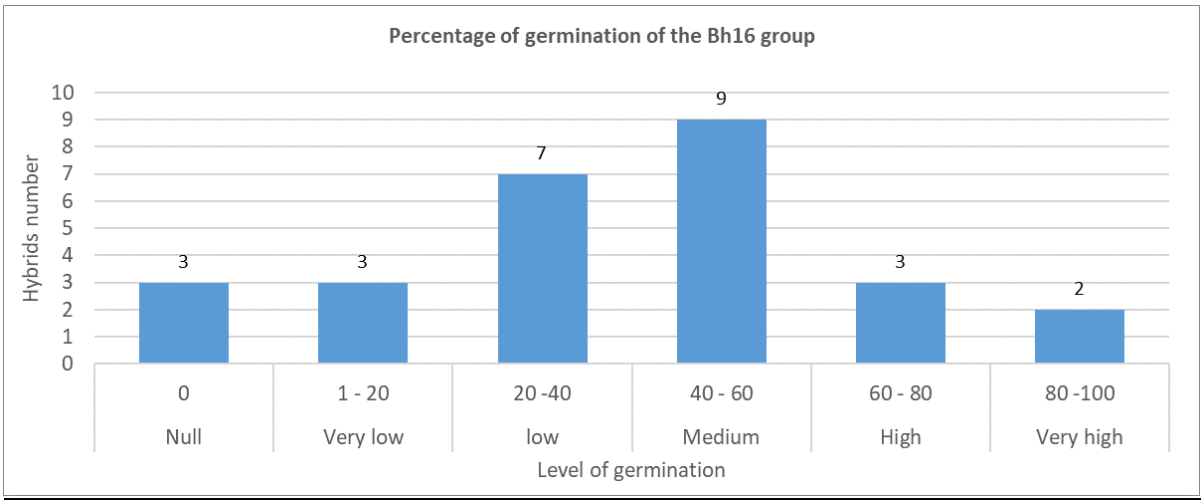
For the sand germination test of each of the Br12 hybrids, the group average is over 23.5%, it was found that 14 hybrids had a germination of 5%, 11 a germination of 10%, 11 a germination of 15 %, 7 a 20%, 13 a 25%, 5 a 30%, 13 a 35%, 8 a 45%, and the remaining 5 a greater than 50%, the material was harvested between August and October 2013 (7 years ago).



Graph 3. Germination percentage of the Br12 hybrid group.

According to graph 3, we see that in general no hybrid of the group exceeds 60% germination, and that most hybrids are at a very low germination level (between 1 and 20%), this can be a inconvenient when establishing hybrids in Mexico if it coincides that low germinations correspond to hybrids that have the least amount of seed, these low levels of germination could be explained with table 1, if one takes into account that the harvest of the entire group It was carried out 7 years ago, because although the longevity of the seeds in optimal storage conditions (Low temperatures and relative humidity) tend to increase, it is known that germination (Herrera, J., 2001.), It has been found that germination of seeds decreases depending on their age (Rivero, L., & Espinosa, J., 1988.), highlighting that this behavior is not recorded for periods of time as long as 7 years.

Regarding the Bh16 group, 6 hybrids had a germination less than or equal to 30%, 8 a germination between 35 and 50% to 50%, 6 a germination between 55 and 70% and the remaining 3 had a germination greater than 70% of the material was harvested between August and September 2017 (3 years ago).



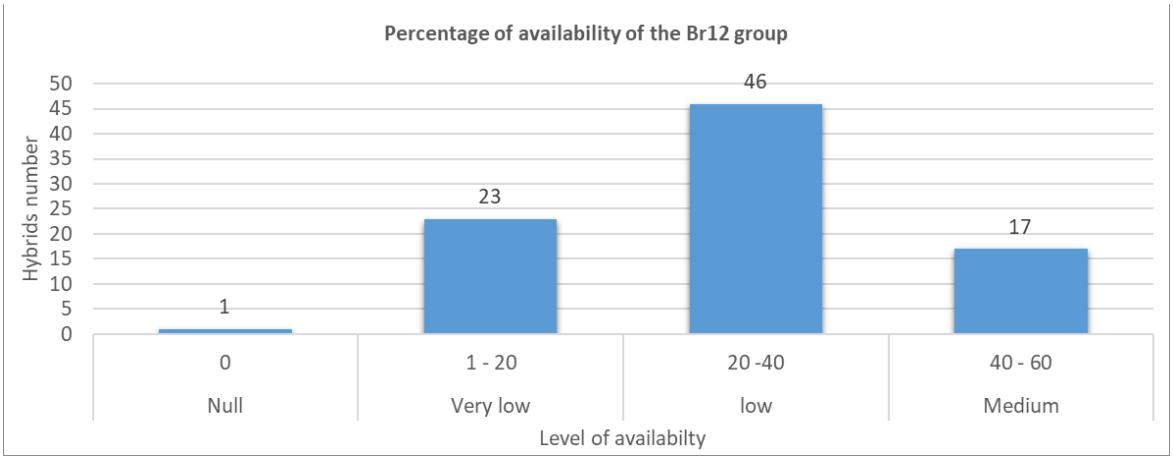
Graph 4. Percentage of germination of the hybrid group Bh16.

For this case, we see in graph 4, that the seeds reach 100% germination percentages, although in general most are located at the medium level of germination (between 40 and 60%), and according to table 1, the

germination percentage of the group is 41.5%, these results are much better compared to those obtained for the Br12 group, in that case the seed only had 3 years of having been harvested, which could indicate that the longevity of These seeds are positioned over 3 years, allowing them to present acceptable germination results after being stored for this time (Herrera, J., 2001.).

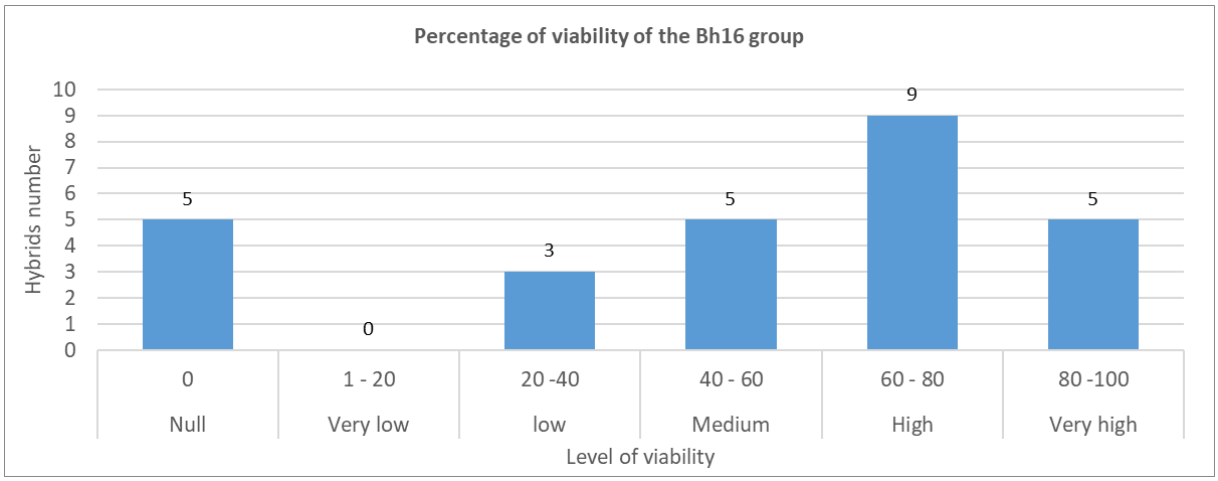
Viability

Regarding the viability, the test was carried out on 86 of the 87 hybrids, with the average being over 31.7%, according to table 1, from this it was found that 1 hybrid presents null or 0% viability, 23 hybrids one out of 1 % and 20%, 46 hybrids have viability between 20% and 40% and the remaining 17 hybrids have a viability greater than 40% but less than 60%. Bh16 Regarding viability, due to the small amount of seed available, only 21 of the 27 hybrids were carried out; from this, 6 hybrids have a viability less than or equal to 50%, 6 hybrids one between 60% and 70%, 7 hybrids have viability between 65% and 85% and the remaining 4 hybrids have a viability greater than 85%.



Graph 5. Percentage of viability of the hybrid group Br12.

En este caso, podemos realizar un análisis similar al que se realizó para germinación, y es que la viabilidad se obtiene al cuantificar mediante el proceso de tinción las semillas que aun esta vivas y presentan procesos metabólicos de respiración (Hernández, G. F. G., Vargas, J. V., & Vázquez, J. L. A., 2007.), tendrán una relación directamente proporcional con la germinación, para este caso el tiempo de almacenamiento aparentemente repercutió negativamente la viabilidad de las semillas como consecuencia de un deterioro natural (Bewley, J. D.; Black, A. M., 1994.).



Graph 6. Percentage of viability of the hybrid group Bh16.

In this case, we can carry out an analysis similar to the one carried out for germination, and that is that viability is obtained by quantifying through the staining process the seeds that are still alive and present metabolic respiration processes (Hernández, GFG, Vargas, JV , & Vázquez, JLA, 2007.), will have a directly proportional relationship with germination, for this case the storage time apparently negatively affected the viability of the seeds as a consequence of natural deterioration (Bewley, JD; Black, AM, 1994 .).

CONCLUSIONS

It is considered that most of the hybrids of the Bh16 collection present good seed quality, except for the 5 hybrids that presented low levels of Viability and Germination (Bh16 / 1771, Bh16 / 2532, Bh16 / 2810, Bh16 / 2567 and Bh16 / 692), in addition, that an important point to improve is the amount of seed available, so it is recommended to continue harvesting to be able to send a larger quantity in subsequent shipments. For its part, the Br12 collection in general does not have very good seed quality, due to its advanced storage time; however, the availability of this will allow the shipment of the 87 hybrids to Mexico.

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